IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: JAMES E. HILDRETH Serial No.: 07/361,271 Filed: June 2, 1989 For: **MONOCLONAL ANTIBODIES AGAINST LEUKOCYTE ADHESION RECEPTOR ß-CHAIN, METHODS OF PRODUCING THESE ANTIBODIES AND USE THEREFOR**	Group Art Unit: 186 Examiner P. Hutzell

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

DECLARATION UNDER 37 C.F.R. §1.132

The undersigned, James E. Hildreth, declares and states that:

1. I am the inventor of U.S. Patent Application Serial No. 07/361,271.

2. I am an Assistant Professor in the Department of Pharmacology and Molecular Sciences at The Johns Hopkins University School of Medicine.

3. I am an expert in the area of leukocyte adhesion receptors.

4. Experiments were performed by me or under my direct supervision and control aimed at determining the unique epitope which is recognized by monoclonal antibody H52.

5. In a first experiment, it was discovered that when H52 is bound to an affinity column and reacted with spleen extracts, only two proteins are retained. The retained proteins are LFA-1α (CD11a) and β subunits (CD18) (Exhibit A, Figure 1, lane 2). When other antibodies, such as MHM.23, are similarly used on affinity columns, all members of the LFA-1 family are retained, including LFA-1α (CD11a), Mac-1α (CD11a), and p150α (CD11c) which are all associated with CD18 (Exhibit A, Figure 1, lane 1). This data shows that H52 has the surprising and unexpected ability to dissociate the Mac-1 and p150-95 subunits, but not the subunits of LFA-1. This unexpected phenomenon has not been reported for any of the other known anti-CD18 antibodies.

6. In a second experiment, a monoclonal anti-idiotype antibody (AIM.6) was produced against the combining site of H52 and tested for binding to a variety of other CD18-specific antibodies. The other CD18-specific monoclonal antibodies that were tested with AIM.6 included P3, 60.3, MHM23, CLB54, M232, TS1/18, 1B4, PLM19, PLM34, PLM22, PLM31, and H5B9. The AIM.6 antibody bound only to H52 and its companion antibody H5B9 (Exhibit B, Figure 9). H52 and H5B9 recognize the same unique epitope.

7. The H52 and H5B9 cell lines have not been publicly distributed.

8. In a third experiment, we established that H52 recognizes the β subunit of CD18 after elution from affinity columns at high pH (Exhibit C, Figure 5). High pH results in the separation of α and β subunits of LFA-1 and it appears that all function-inhibiting anti-CD18 antibodies except H52 no longer bind to CD18 after separation of the subunits.

9. The results of the above-noted experiments would lead one of skill in the art familiar with the known monoclonal antibodies to CD18 to the unexpected and surprising conclusion that H52 recognizes a unique epitope on CD18.

9. The significance of the unique properties of H52 are further supported by the fact that an exclusive license to this monoclonal antibody has been granted to a major biotechnology pharmaceutical company to assist in the development of products which will be of benefit to our society.

10. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made, are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 5/29/91

James E. Hildreth